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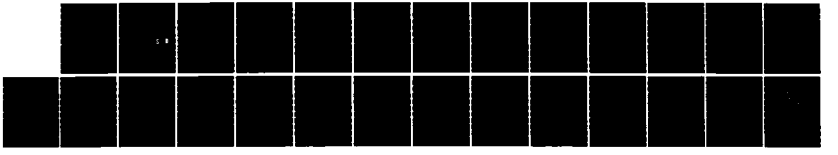

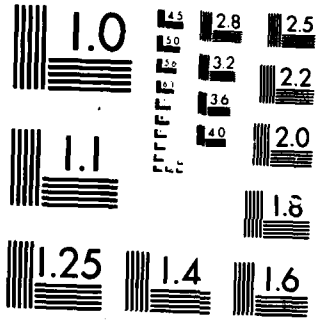

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OFFICE OF NAVAL RESEARCH

Contract No. N00014-75-C-0340 P0003

Task No. NR 133-081

FINAL REPORT

Microbial Ecology Studies of Biofouling of Treated and Untreated Wood Pilings in the Marine Environment

by

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28 February 1986

Distribution of this report is unlimited.

ABSTRACT

The role of marine microorganisms in facilitating attachment, invasions, and subsequent destination, viz., biofouling, of solid substrata, especially wood, in the marine environment was studied. Relationships between microorganisms, primarily bacteria, and invertebrate macroorganisms that bore into wood were also investigated. It was concluded that naphthalene enrichment of creosote retards biofouling of wood pilings during the first year of placement of the treated wood into tropical marine waters. After that, little or no slowing of biofouling can be detected. The wood boring invertebrate, Limnoria, appears to develop a gut microflora that is both naphthalene resistant and capable of naphthalene degradation. It was hypothesized that this gut microflora enabled the wood-boring Limnoria to survive on creosote/naphthalene-treated wood. Ames assay revealed no detectable mutagenicity in seawater samples collected near creosote/naphthalene-treated pilings.

OBJECTIVES

The research program proposed by the University of Maryland and supported by the Office of Naval Research by means of Contract No. N00014-75-C-0340 P00003 had as a primary objective evaluation of naphthalene-enriched creosote as a treatment for preservation of wood pilings in the marine environment. Specific objectives were: (1) to compare the rate and extent of microbial fouling of wood pilings with and without treatment, i.e., without treatment, with standard marine-grade creosote treatment, and with 10, 20, 30 and 40% naphthalene-creosote treatment; (2) to gain an understanding of the microbial ecology of the biofouling process using methods developed and employed in previous work supported by the Office of Naval Research, for enumeration, identification, and metabolic analysis of the microorganisms involved in the biofouling process; (3) to determine the association, if any, of the biofouling microorganisms and the wood borer, Limnoria tripunctata; (4) to measure the environmental impact, if any, on the water and sediment of the area in which the naphthalene-enriched creosote-treated wood pilings were installed, including leaching of the naphthalene-enriched creosote, and effects on the microbial populations, as determined by in situ studies combined with laboratory experiments; (5) to determine seasonal effects on the microbial populations and the relationships of these effects to the biofouling processes using Pier #1, the fueling pier, and Pier #3 as the sites for comparison; and (6) to provide an evaluation of naphthalene-enriched creosote treatment of wood pilings as a marine preservative and, subsequently, a recommendation for wood piling treatment for the naval facility at Roosevelt Roads, Puerto Rico.

BIODEGRADATION - BACKGROUND

Research work on marine microbiology, microbial ecology and microbial aspects of oil biodegradation has been underway at the University of Maryland since 1972. In the earlier phases of work supported by the Office of Naval Research, psychrophilism, barophilism, ultrastructure, identification and classification of marine microorganisms were studied and a series of publications resulted.

The basic research findings from our laboratory have been applied to the problem of biodegradation of oil and biodeterioration of wood and other materials in the marine and estuarine environment. Sediment samples from the continental slope and trenches of the Atlantic Ocean have been studied, along with Chesapeake Bay and Roosevelt Roads, Puerto Rico water and sediment samples. Petroleum degradation under deep-ocean conditions and mediated by marine bacteria has been described. Radioactively labeled hydrocarbons, serving as substrate for our deep-ocean isolates, were employed in experiments which showed conclusively that hydrocarbon utilization by microorganisms does occur under simulated conditions of deep-ocean pressure and temperature.

A series of Chesapeake Bay studies has been carried out. Among the results reported in publications arising from research work supported by this contract are the following. Aerobic heterotrophic microorganisms present in Chesapeake Bay sediment and water were enumerated, isolated, and described. A complete annual cycle of the incidence of selected generic and physiological groups of bacteria was also described, including petroleum-degrading microorganisms. Hydrocarbon composition of sediment and

water samples were determined and the pathways of hydrocarbon metabolism in selected marine and estuarine bacteria isolated from Chesapeake Bay, Puerto Rico, and Atlantic Ocean coastal zone samples studied. As a result of our research work, it is clear that bacteria in the water column of Colgate Creek, in Baltimore Harbor of Chesapeake Bay, and San Juan Harbor, Puerto Rico, are capable of active biodegradation of several crude oils, including South Louisiana crude oil, whereas bacteria in the relatively unpolluted water and sediment do not carry out as complete a biodegradation of the oils. Computerized gas-liquid chromatography and low-resolution mass spectrometry have been employed in these analyses.

The interaction of heavy metals, pesticides, and other pollutants with oil has been studied and we have discovered that heavy metals can concentrate in the oil phase of a water-sediment-oil mixture, such that the growth of some petroleum-degrading microorganisms will be inhibited. The interactions of selected pollutants in the marine and estuarine environment are of great significance in evaluating environmental impact.

Another aspect of the work accomplished with support from the Office of Naval Research is the determination of the effects of petroleum and petroleum products on the natural microbial flora of the estuarine and marine environment. Several physiological groups of bacteria were studied, including chitin-digesting, proteolytic, amylolytic, and lipolytic bacteria in water and sediment. The population numbers of these bacteria in sediment and water samples exposed to oil did not reach population levels of samples not exposed to oil. Thus, petroleum can limit bacterial growth, with measurable effects of oil on ecologically important bacterial groups. The crude and refined oils examined in our studies demonstrated significant

limiting effects on total viable numbers and on the activities of ecologically important bacterial groups. In a related study, not funded by the Navy (Zachary et al., 1978) we showed that scanning electron microscopy (SEM) could be used to evaluate materials for susceptibility to biofouling. We also confirmed that microbial colonization of surfaces follows a successional pattern, in which bacteria are the first to attach (Zachary et al., 1978).

BIODEGRADATION OF NAPHTHALENE

During 1976-1978, work in our laboratory concerned naphthalene degradation and the effects of naphthalene on microorganisms of ecological significance. Naphthalene can serve as a growth substrate for a great variety of microorganisms and its pathway of degradation has been studied in detail by several investigators (Dean-Raymond and Bartha, 1975). Recently, utilization of several polycyclic aromatics, including naphthalene, has been shown to be under control of plasmid-borne genes (Chakrabarty, 1980; Kopecko, 1980). However, the microbial ecology aspect of naphthalene degradation is relatively neglected. Thus, several experiments were undertaken to determine the extent and rate of degradation of naphthalene, creosote with 10% naphthalene, and creosote with 40% naphthalene. Sampling areas studied were Colgate Creek, Eastern Bay, and Little Creek, all in Chesapeake Bay. The total heterotrophic bacteria, yeasts and fungi were monitored, along with cellulolytic, chitinoclastic and proteolytic bacteria. Experiments, whereby the various naphthalene-containing mixtures were exposed in flask culture to water and sediment from each of

the sampling areas, permitted estimation of naphthalene biodegradation and effects on the natural microbial populations.

BIODETERIORATION OF WOOD

As an extension of this work, the field study was expanded to include the Roosevelt Roads, Puerto Rico, Naval Base site, since biodegradation of wood pilings at Roosevelt Roads is a serious problem. Water samples were collected at Pier 3 at Roosevelt Roads in February 1976 and at quarterly intervals thereafter. Water and wood samples were collected and examined for total viable, aerobic, heterotrophic microorganisms and for microorganisms capable of growth on naphthalene, creosote, and creosote containing 40% naphthalene. Wood samples were also examined for wood-boring organisms. The wood borers were extracted, pooled, homogenized, and plated on agar to enumerate the associated bacteria.

Coupons of creosote and naphthalene/creosote treated wood were exposed to the water near the pilings for five days. Thereafter, the coupons were collected, fixed, and prepared for electron microscopy in order to determine whether a primary film layer forms on the wood surface.

From preliminary results of the field studies carried out at Roosevelt Roads, Puerto Rico during 1976-1978, it became clear that, after submergence for four days, wood surfaces were heavily colonized by microorganisms, despite the low numbers of microorganisms present in seawater at the site. The presence of naphthalene and of naphthalene-treated creosote appeared to delay microbial attachment and biofouling. From SEM observations, a reduction in the rate of microbial colonization of wood surfaces was associated with increased naphthalene content of creosote-treated wood.

Taken in conjunction with results showing an inhibitory effect of naphthalene on agar-digesting microorganisms comprising microbial biofouling populations, naphthelene appears to play at least an initial role in protecting wood from biodegradation. The selective effects of naphthalene observed in the field studies are consistent with the selective effects of naphthalene observed in experiments undertaken in the laboratory, utiliizing seawater samples collected at Roosevelt Roads, to which naphthalene-creosote mixtures and naphthalene were added. However, leaching of the naphthalene-creosote mixture appears to occur, with the subsequent result that, after several months, biodeterioration occurs at nearly the same rate as for creosote-treated wood without naphthalene added. Most of this work was published in 1980 (Colwell et al., 1980).

The microbial counts of the water, sediment, and wood samples collected at Roosevelt Roads, Puerto Rico, at Pier No. 3, showed that the largest microbial populations were associated with the wood pilings, compared with water and sediment; i.e., there were two and four orders of magnitude greater microbial populations in the sediment and wood, respectively, compared with the surrounding water. At the site under study, bacteria may prove to be more active in biodeterioration of cellulose than the fungi (Jones, 1971), since relatively few fungi were detected, compared with the populations of cellulolytic bacteria found associated with the wood pilings. Dominant data in the water, sediment, and piling samples included Hyphomicrobium, Hyphomonas, Pseudomonas, Vibrio, and Bacillus. New wood exposed to harbor water was rapidly colonized by Hyphomicrobium vulgare. Oil pilings, in an advanced stage of biodeterioration, maintained a diverse bacterial microflora, representatives of which were also found

widely distributed in the water column and sediment. Evidence for bacterial species succession was obtained, indicating that microbial interactions are important for attachment to, and subsequent colonization of, wood surfaces in the marine environment (Austin et al.. 1979).

In December, 1979, water, sediment and wood piling samples were again collected at the Roosevelt Roads Naval Station. Results indicated no detectable traces of naphthalene in water or sediment samples collected at the control site at Puerto Rico as measured by gas-liquid chromatography; only traces could be detected in sediment at Pier No. 3. Naphthalene was not detected in the wood at the top of the old pilings and only trace levels were found in the top wood from new pilings. An increase in naphthalene content was observed in both new and old pilings from the top of the piling to the intertidal level and to the bottom of the piling at the water-sediment interface.

Beach sand at the December, 1979 control site yielded only Gram-positive, fermentative rods identified as Bacillus spp. At Pier No. 3, new wood yieldeo only Gram-negative, stalked rods that were not capable of degrading casein or starch, unable to use tyrosine and serine, or hemolyze blood and were penicillin sensitive. These were identified as Hyphomicrobium sp. The bacteria trom old wood were Gram-negative rods that were hemolytic, and proteolytic, and capable of degrading starch and casein and utilized agrosine and serine and were penicillin resistant. The sediment samples contained a taxonomically diverse community. The microorganisms colonizing the new wood pilings were predominantly Hyphomicrobium and halt were naphthalene degraders. The old pilings possessed a microflora reflective of the water and sediment.

Scanning electron microscopy showed that microfouling was rapid, with rate and extent of fouling of untreated wood being significantly greater than for naphthalene/creosote treated wood; no differences were noted in rate of attachment to 10 and 30% naphthalene/creosote treated piling. However, the kinds of bacteria involved in attachment were different for the control, compared with napththalene/creosote treated wood. Examination of L. tripunctata from the pilings by transmission electron microscopy revealed the presence of extensive sessile bacterial communities in the tunnels and on the exoskeleton surfaces of the borer, an observation that was recently confirmed by Boyle and Mitchell (1981). These bacteria are being studied to determine capability to fix nitrogen. The isopod gut was found to contain bacteria and this gut microflora was tested for hydrocarbon utilization. It is hypothesized that a commensal relationship exists between the gut-associated bacteria and L. tripunctata, perhaps contributing to creosote-resistance of the wood borer. Interestingly, L. tripunctata collected from untreated wood, or reared on untreated wood in aquaria, do not contain gut microorganisms (Zachary and Colwell, 1979). In contrast, the digestive tract of L. tripunctata inhabiting creosote-preserved wooden pilings possesses a unique, membrane-associated microflora that was observed to contain numerous electron transparent cytoplasmic inclusions, consistent with those observed in hydrocarbon grown bacteria (Zachary and Colwell, 1979).

BACTERIAL ATTACHMENT

Interaction of marine bacteria with solid substrata was examined in the course of these studies. It was discovered that adsorption of laterally and polarly flagellated bacteria could be described by a modified Langmuir adsorption isotherm. Laterally flagellated Vibrio parahaemolyticus followed the Langmuir adsorption isotherm, a type of adsorption referred to as surface saturation kinetics, when conditions were favorable for production of lateral flagella. When conditions were not favorable for production of lateral flagella, bacterial adsorption did not follow Langmuir kinetics; instead, proportional adsorption kinetics were observed. Results therefore indicate that laterally flagellated bacteria adsorb according to surface saturation kinetics, whereas polarly flagellated bacteria adsorb by proportional adsorption kinetics (Belas and Colwell, 1982a). In a related study (Belas and Colwell, 1982b), V. parahaemolyticus was observed to swarm across solid agar surfaces, by virtue of forming heavily flagellated (lateral and polar), elongated swarm cells.

RECENT FIELD STUDIES

Sampling of water, sediment, and wooden pilings at the Roosevelt Roads Naval Station was conducted in February 1982. Preliminary results indicate that the effectiveness of naphthalene-enriched creosote in delaying fouling and preventing borer infestation substantially diminished, since installation of the pilings in 1978. Both 0% and 40% enriched pilings evidenced extensive deterioration in the intertidal zone, and samples demonstrated penetration of limnoria to depths > 4 cm. In contrast to prior samplings, total aerobic heterotrophic counts from treated, untreated, or control

wood were similar. Direct spread-plate enumeration of bacteria from wood on various selective media showed no substantial differences related to preservative treatment. While not seasonally adjusted, data from two prior sampling trips normalized to sediment counts, which have remained stable, showed sequential increases of two and three orders of magnitude in total counts from 40% naphthalene-enriched creosote treated wood. Additionally, preliminary findings indicate greater taxonomic diversity in the wood associated bacteria than had been observed previously.

Higher numbers and morphological heterogeneity also characterized the organisms detected in intimate contact with limnoria. Most recent total counts on homogenates were on the order of 10^5 per limnoria as opposed to 10^3 and 10^1 in 1981 and 1979, respectively. As with the wood samples from which the limnoria were obtained, no differences in preservative treatment were reflected in any of the observed counts. A large proportion of the total counts on whole limnoria were also culturable on a medium selective for Vibrio species and on one selective for Aeromonas species, suggesting predominance of these bacteria. As had been noted previously, drastic changes in the limnoria flora occur upon transfer of specimens to a laboratory environment.

AQUARIUM STUDIES OF LIMNORIA

Samples of control, standard marine creosote, and 40% naphthalene-enriched creosote treated wood, each inhabited by limnoria, were maintained in separate tanks at our laboratory. Aquaria for holding specimens were established with appropriate aeration, temperature,

illumination and salinity conditions. Water consisted of 40% synthetic marine salts mix, 40% activated carbon absorbed natural sea water and 20% in situ water. A closed filtration system was "pre-cycled" to obtain acceptable ammonia and nitrite-nitrogen levels before introduction of the limnoria. Samples were kept in a humid, temperature-controlled environment during the 8-hour transit from Roosevelt Roads harbor. Despite maintenance of stable conditions, the natural flora of limnoria was not preserved.

Within one week, total and Vibrio numbers had declined two orders of magnitude. Limnoria gut-associated organisms were not detected on any of several media, with one exception. On naphthalene plates, which had been inoculated with UV-surface-sterilized limnoria homogenate, minute colonies were detected. Why dissected digestive tracts or unirradiated limnoria did not yield similar organisms remains unclear, as does the reason for decline and changes in other associated flora. It is evident that conclusions drawn from laboratory-reared specimens may not completely reflect conditions in nature, and demonstrates the necessity of direct field studies.

PAH ANALYSES

Numerous polycyclic aromatic hydrocarbons (PAH) are known to be mutagenic and/or carcinogenic for many eucaryotic and procaryotic organisms and probably for man. Introduction of such compounds into the aquatic environment should be carefully considered, with regard to ecological implications and environmental consequences. Natural volcanic activities and marine processes involving microorganisms, phytoplankton, algae, and plants are responsible for a significant input of PAHs into the sea

(Andelman and Suess, 1970). Major influxes from disposal, leaching runoff and fallout of PAHs, however, are a direct result of anthropogenic activities, especially severance, transport, pyrolysis, and combustion of hydrocarbon deposits. Of particular concern in this study was the purposeful exposure of the marine ecosystem to PAHs via creosote-treated wood pilings. Because of speculation that tumors in marine animals may be induced by PAHs (Dun and Stich, 1976; Matsushima and Sugimevea, 1976; Barry and Yevich, 1975; Brown et al., 1977; Mix et al., 1979; Kraybill, 1977; Gardner, 1975), it is clear that there is a need for monitoring the presence of mutagens.

Creosote has been estimated by the Environmental Protection Agency to be the largest volume pesticide used in the United States (Science/Technology Concentrates, 1978), with application estimates of 10 pounds per year. Creosote is a complex mixture of at least 160 detectable hydrocarbon compounds, of which all 18 major components are cyclic and aromatic (McNeil and Vaughn, 1964). The fate of these PAHs in the sea is linked to several natural mechanisms, namely, evaporation, dispersion, emulsification, photochemical oxidation, dissolution, adsorption to sediments, biological degradation, and concentration in the biota. Our studies were directed toward monitoring the effects of sediment adsorption, degradation and bioconcentration of potentially mutagenic PAHs in creosote used in treating pilings for protection against biofouling.

While factors such as intensity and duration of illumination, warm temperatures and high dissolved oxygen concentrations favor hydrocarbon photo-oxidation (Andelman and Suess, 1971), in the harbor area where this study is being carried out, cyclic and fused ring structures, such as PAHs

in creosote, are the most resistant to such degradation, as well as being, in general, the least soluble, and, consequently, the most likely to persist in the environment. Microbial degradation involves the simplest and least toxic alkanes primarily (Petroleum in the Environment, 1975). Naphthalenes and alkylnaphthalenes, the major soluble toxic components (Anderson et al., 1974) are degraded more slowly (Dean-Raymond and Bartha, 1975), followed by the relatively inert PAHs which may be ingested and passed up the food chain. Roubal et al. (1977) presented evidence suggesting that the degree of accumulation of PAHs by fish is proportional to the number of fused rings. Higher marine organisms have been shown to possess the necessary aryl hydrocarbon hydroxylases (AHH) and other enzymes required to metabolize PAHs (Lee et al., 1972a; Corner et al., 1973; Sanborn and Malins, 1977; Payne, 1977). Such enzymes can activate PAHs to produce proximate mutagens and carcinogens. Lee et al. (1972b) have shown that mussels do not possess AHHs and can accumulate PAHs during filter-feeding. If biomagnification of PAHs occurs in marine ecosystems, sampling of filter-feeding molluscs for compounds capable of inducing mutation, when the necessary enzyme systems are present, should provide a useful index of safety.

MUTAGENICITY STUDIES

Mutagenic potential can be assayed in shellfish using the procedures of Ames et al. (1975) and extraction procedures developed for molluscs, described by Parry et al. (1976). In this study, several extraction schemes were considered, including soxlet, serial liquid-liquid extractions and saponification (Cahnmann and Kuratsane, 1957), with adjunct

chemical, adsorption chromatographic (Yamasaki and Ames, 1977) or high pressure liquid chromatographic separations. However, many of the solvents employed in extractions for chemical analyses of PAHs are themselves mutagenic under certain conditions, notably CCl_4, benzene, chloroform, methylene chloride, dioxane, aniline, ethylene chloride, and tetrahydrofuran, while others are highly reactive and/or dangerous to handle. In addition, fractionation of sample extracts presumed to contain creosote components or their photochemically or biologically altered products would be unnecessarily complex and detract from the main objective of the application of the Ames assay: the ability to detect mutagens in a complex mixture without component resolution (McCann and Ames, 1976). When such resolutions are carried out, the sum of all activities in the fractions does not necessarily equal the activity of the initial extract. Some fractions contain enhancing or inhibitory properties, or display synergistic interactions. Our objective was to obtain an approximation of the total mutagenic activity of the samples resulting from exposure to naphthalene-enriched creosote and creosote. Simple ethanolic extractions were therefore employed in our laboratory for samples collected from Chesapeake Bay and these proved satisfactory for detection and quantitation of mutagenic activities, without complex and time-consuming extractions.

The Ames test (Ames et al., 1975) employs strains of Salmonella typhimurium which are mutant in the ability to synthesize the amino acid histidine (His^-). Additionally these strains are defective in cell wall lipopolysaccharide, increasing their permeability to large compounds, and are also defective in a DNA excision repair system, which makes them more sensitive to most mutagens. The strains mutate at a low spontaneous rate

to histidine independence (His^+). The number of cells which have undergone reverse mutation to His^+ (revertants) is easily determined by plating large numbers of cells on solid medium lacking histidine, where only the few revertant cells can grow and form colonies. Mutagenicity of extracts is detected in the test by their ability to induce His^+ revertants over that which occurs spontaneously.

Samples were collected on January 14, 1979, near Pier 3 of the Roosevelt Roads Naval Station Complex, Puerto Rico. Oysters and clams were collected off the bottom at Pier 3 at a depth of approximately 10 meters and within 10 meters of the pilings. The pilings in place, alternatively positioned, have been treated with 10, 30, and 40% naphthalene-enriched creosote (Koppers Co.).

The shellfish were immediately shucked upon collection. In addition, water and sediment at Pier 3 were collected at the base of a 40% naphthalene-creosote treated piling at the same depth. Water and sediment samples were also collected about 2 kilometers from Pier 3, near a mangrove swamp, and served as controls. Because diving conditions at the time of sampling were hazardous, no molluscs at the control site were obtained. All samples were promptly frozen (~ - 20 C) and were treated by three freeze-thaw cycles during transport to the laboratory for processing.

Assays were performed using the plate incorporation method of Ames et al. (1975), with the additional recommendations of deSerres and Selby (1978). All plate assays were run in duplicate, with sample, enzyme, and reaction mixture sterility tests, and positive and negative controls included. Total solvent concentration was held constant on each plate by addition of solvent to a fixed total volume. Arochlor 1254-induced

rat liver homogenate (RLH), obtained from Litton Bionetics, Kensington, Maryland, had a Benzo (α) pyrene hydroxylase activity of 0.635 nmol hydroxybenzpyrene/20 minutes/mg protein.

One-hundred and fifty µl of 9000G-supernatant per plate in the S-9 core reaction mixture were employed. This volume was found to provide maximal reversion with the tester strains, TA98 (a system which detects base substitutions) and TA100 (which detects frameshifts) when exposed to Benzo (α) pyrene, i.e., the positive control.

Extracts of "clean" control sediment induced the greatest increases in revertants, and an oil spill which occurred near the control area prior to sampling appears to have contaminated the samples. The linear response of only one tester strain to these extracts suggests the persistence of a more homogeneous and active mutagenic component from the control sediment than from Pier 3. Extracts of samples collected at Pier 3 showed less linear, and more variable, dose dependent activity with both strains. Such a response is not unexpected, since not only the mutagen source in question, creosote, is an unresolved mixture, but so are the RLH enzymes. As dose levels are increased, the ratio of enzymes, i.e., activating AHH or competing enzymes, including epoxide hydratases, and gluthathione-s-transfer-ases (Ashby and Styles, 1978), to substrates is altered and cannot be optimized when both variables are in flux and neither is defined. However, by consistently employing the same enzyme preparation and reaction volume, dose-dependent responses can be detected within more closely defined ranges and comparisons with suitable controls are valid.

The capability of the system employed in this study to detect mutagens in sediment is consistent with results of previous work in our

laboratory. Sediment binding of hydrocarbons must be considered, since this limits access to photooxidation in the aquatic environment and contributes to PAH persistence.

Water samples yielded no detectable activity. The low solubility of creosote in water and weather conditions favoring evaporation, dispersion and photooxidation of the creosote probably explain its absence from the water column. Accumulation of mutagens in the mollusc samples was not observed. Other authors have detected components of creosote in mussels (Dunn and Stich, 1976), clams, and periwinkles (Eaton and Zitko, 1978) by chemical methods. However, the levels detected are dependent on the age of the piling and the proximity of molluscs to it. As pilings age, the more toxic components leach out or are degraded. Succeeding biological communities may include molluscs that are in closer proximity to, or in contact with, the pilings. Eaton and Zitko (1978) noted that, while PAH levels in clams and mussels were two orders of magnitude below those detected in sediment, periwinkles, some of which were in direct contact with the wharf and may have ingested creosote, contained intermediate PAH concentration.

The Ames test was again employed to assess the presence of creosote in the February, 1982 samples. There was no detectable increase in the number of revertants, leading us to conclude that creosote does not exhibit any appreciable leaching into surrounding water.

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